FORM PTO-1390 (REV 11-98) | U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
1012-101US	09/622253

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/00385	February 5, 1999 (05.02.99)	February 16, 1998 (16.02.98)

TITLE OF INVENTION: DERIVATISED ANTIBODIES WITH EXPOSED CARBOHYDRATE CHAINS CAPABLE OF BINDING TO IMMOBILISED IGG

APPLICANT(S) FOR DO/EO/US: RADEMACHER, Thomas William and Williams, Phillip

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (unsigned)
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:

Please note changes have been made to the International specification and claims under Article 34. The following claims fees have been calculated based on the claim set attached to the PCT IPER as well as the amendments presented in the attached Preliminary amendment.

The following are also included:

- International Preliminary Examination Report dated February 17, 2000
- Verified Statement of Small Entity Status
- Acknowledgement of Receipt Postcard
- Certificate of Mailing

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/622253	PCT/GB99/00385	1012-101US

	CALCULATIONS PTO USE ONLY	
17. [X] The following fees are submitted:		
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$970.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$840.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$760.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$670.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$96.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 840	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	23 - 20 =	3	X **$18.00**	$ 54	
Independent claims	3 - 3 =	0	X **$78.00**	$ 0	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$260.00**	$	
TOTAL OF ABOVE CALCULATIONS =				$ 894	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also by filed (Note 37 CFR 1.9, 1.27, 1.28).				$ 447	
SUBTOTAL =				$ 447	
Processing fee of **$130.00** for furnishing the English translation later than [ ]20 [ ]30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 447	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 447	
				Amount to be: refunded	$
				charged	$

a. [ ] A check in the amount of $_____ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 50-0893 in the amount of $447 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-0893. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Jonathan Alan Quine
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SIGNATURE:

Jonathan Alan Quine
NAME

41,261
REGISTRATION NUMBER

I hereby certify that this is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above, addressed to: Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231

By: Andrew Merit
Andrew Merit

Attorney Docket No. 1012-101US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: RADEMACHER, Thomas William, et al. Application No.: (not yet known) Filed: (Submitted herewith) For: DERIVATISED ANTIBODIES WITH EXPOSED CARBOHYDRATE CHAINS CAPABLE OF BINDING TO IMMOBILISED IGG	Examiner: Unassigned Art Unit: Unassigned PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-referenced application, please enter the following amendments and remarks.

IN THE CLAIMS

Please amend the claims as follows. The Amendments merely conform the claims to U.S. practice with respect to claim dependency issues and introduce no new matter. Support for the amendments is replete throughout the specification and claims as filed.

3 (Amended). The method of claim 1 [or claim 2], wherein the method includes the step of using B-galactosidase to remove terminal galactose residues from the precursor antibody.

4 (Amended). The method of [any one of claims 1 to 3] claim 1, wherein the chemical derivatisatinq step comprises thiolating the antibody in the presence of carbonate.

5 (Amended). The method of claim 1 [any one of the preceding claims], wherein the method includes the step of separating derivatised antibodies which can associate with one

another at a site of immobilised IgG, from those derivatised anti-bodies which cannot so associate.

7 (Amended). An antibody as obtainable by the method of claim 1 [any one of the preceding claims], wherein the antibody has been derivatised to expose a carbohydrate chain thereof so that the antibody is capable of binding to immobilised IgG.

9 (Amended). The antibody of claim 7 [or claim 8], wherein the carbohydrate chain terminates with an N-acetylglucosamine residue.

10 (Amended). The antibody of [any one of claims 7 to 9] claim 7, wherein the carbohydrate chains of the derivatised antibody are capable of specifically binding a binding site on the immobilised antibody.

11 (Amended). The antibody of [any one of claims 7 to 10], claim 7, wherein the immobilised IgG is agalactosyl IgG.

12 (Amended). The antibody of [any one of claims 7 to 11] claim 7, wherein the antibody is conjugated to a label, toxin, drug, prodrug or effector.

13 (Amended). The antibody of [any one of claims 7 to 12] claim 7, wherein the antibody is specific for an inflammatory mediator.

14 (Amended). The antibody of [any one of claims 7 to 13] claim 7 for use in a method of medical treatment or diagnosis of a condition associated with immobilised IgG.

18 (Amended). Use of a derivatised antibody of [any one of claims 7 to 14] claim 7 for the preparation of a medicament for the treatment or diagnosis of a condition associated with immobilised IgG.

22 (Amended). Use of a derivatised antibody of [any one of claims 7 to 14] claim 7 for the in vivo diagnosis of a condition associated with immobilised IgG, wherein the use comprises:

(a) exposing a Patient to said antibody which has been labelled; and,

(b) using the label to detect the presence of the immobilised IgG.

23 (Amended). A pharmaceutical composition comprising a derivatised antibody of [any one of claims 1 to 14] claim 1, in combination with a carrier.

CONCLUSION

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 510-337-7871.

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Respectfully submitted,

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Reg. No. 41.261

Atty. Docket No. 1012-104US

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27(c)) - SMALL BUSINESS CONCERN

Applicant or Patentee: RADEMACHER, Thomas William and WILLIAMS, Phillip
Application or Patent No.: (not yet known)
Filed or Issued: (submitted herewith)
Title: DERIVATISED ANTIBODIES WITH EXPOSED CARBOHYDRATE CHAINS CAPABLE OF BINDING TO IMMOBILISED IGG

I hereby declare that I am:

- ☐ the owner of the small business concern identified below;
- ☒ an official of the small business concern empowered to act on behalf of the concern identified below:

Name of Small Business Concern: University College London
Address of Small Business Concern: Gower Street, London WC1E 6BT United Kingdom

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled **DERIVATISED ANTIBODIES WITH EXPOSED CARBOHYDRATE CHAINS CAPABLE OF BINDING TO IMMOBILISED IGG** by inventor(s) **RADEMACHER, Thomas William and WILLIAMS, Phillip** described in:

- ☒ the specification filed herewith.
- ☐ Application No. _, filed .
- ☐ Patent No. ______, issued ______.

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights in the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern that would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

Name ______
Address ______

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

Name ______
Address ______

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing: ______
Title of Person if Other than Owner: ______ for University College London
Address of Person Signing: Gower Street, London WC1E 6BT, United Kingdom

Signature ______ Date 14 AUGUST 2000

J.D. Skinner
Director
UCL Ventures

DERIVATISED ANTIBODIES WITH EXPOSED CARBOHYDRATE CHAINS CAPABLE OF BINDING TO IMMOBILISED IGG

Field of the Invention

The present invention relates to derivatised antibodies and their use in the diagnosis and treatment of conditions associated with immobilised IgG, specifically autoantibodies, and in particular to conditions associated with elevated levels of immobilised IgG, such as rheumatoid arthritis (RA). The present invention also relates to a method of producing the derivatised antibodies.

Background of the Invention

A central problem in clinical rheumatology is the need to define at presentation using non invasive techniques those patients who will go on to develop erosive joint disease. If such patients can be identified, then aggressive therapy can be targeted to them and more conservative therapy can be reserved for the remaining patients with RA who do not go on to erosive joint disease. This would improve the treatment of both groups, since early treatment in the erosive group can prevent the progression of disease and in the benign group reduce morbidity due to less frequent and severe drug reactions and toxicity.

A number of clinical parameters have been assessed for their ability to predict outcome of erosive joint disease. These include age, sex, RF status, genetic background (HLA) and serum agalactosyl IgG levels (see for example JP-A-5087814). However, prior studies have found that no single parameter is useful as a predictor. However, a combination of agalactosyl IgG levels, age of

onset, gender, functional assessments and RF titre predicts the course of RA correctly in 94% of patients.

Agalactosyl IgG is an IgG glycoform which is increased in patients with rheumatoid arthritis, tuberculosis, Crohn's disease, juvenile onset arthritis, sarcoidosis, type I insulin dependent diabetes, type II diabetes and a form of leprosy called erythema nodosum leprosum. Agalactosyl IgG is thought to cause pathology in the respective target tissues by a variety of mechanisms including:

(a) Tissue damage via the interaction of *in situ* immobilised agalactosyl IgG and the lectins mannose binding protein (MBP), tumour necrosis factor (TNFα) and transforming growth factor (TGFβ). The tissue immobilised autoantibodies can bind these inflammatory mediators and trap them in the tissue, leading to tissue damage.

(b) Agalactosyl IgG is thought to cause systemic pathology by forming self-associating immune complexes.

The target organ or tissue for agalactosyl IgG action depends on the specificity of the autoantibody. In the case of rheumatoid arthritis, it is thought that autoantibodies against type II collagen are targeted to the joint. Cross-sectional studies of rheumatoid arthritis patients have demonstrated a frequency of only 30% for patients with detectable antibodies to type II collagen late in disease. In early RA the frequency may reach 70%. The problem of identifying patients likely to

develop erosive joint disease is complicated as three groups of rheumatoid arthritis patients can be classified by their anti-collagen autoantibody serum titres.

Group 1: Normal titre of anti-type II collagen antibodies.

Group 2: Elevated titre of anti-type II collagen antibodies which remain constant.

Group 3: Elevated titre of anti-type II collagen antibodies early in disease with a decrease to normal level later in disease. In this case, the decrease in the level of serum antibodies to collagen may include:

(a) Intrasynovial synthesis of antibodies to collagen (in contrast to systemic production);

(b) Removal of antibodies to collagen secondary to immune-complex formation; and/or,

(c) Serum skimming by immobilisation of antibodies to damaged cartilage surfaces.

Of the three groups described above, Group 3 will go on to get severe erosive disease and Group 1 mild disease. Therefore, serum antibody levels cannot discriminate between Group 1 and 3 once the levels of antibody in Group 3 has returned to normal.

While, the affected synovial joints of Group 3 patients

(in contrast to Group 1) are coated with anti-type II collagen antibodies of the agalactosyl IgG glycoform, joint biopsy is not a routine nor desirable procedure to detect these antibodies.

Summary of the Invention

Broadly, the present invention provides derivatised antibodies, the antibodies being derivatised to expose carbohydrate chains so that the antibodies are capable of binding to immobilised IgG, such as agalactosyl IgG. Thus, the derivatised antibodies can be employed in the diagnosis and treatment of conditions associated with immobilised IgG, such as rheumatoid arthritis (RA) (Group 3 versus Group 1 above). In particular, the present invention provides a non-invasive technique which can detect the immobilised IgG *in situ*, thereby assisting in the diagnosis or treatment of conditions associated with it. The present invention can help to avoid measuring the range of clinical parameters currently used to determine the likely course of RA.

Accordingly, in a first aspect, the present invention provides a derivatised antibody, the antibody being derivatised to expose carbohydrate chains so that the antibody is capable of binding to immobilised IgG, e.g. agalactosyl IgG. Preferably, the derivatised antibodies are also capable of self associating or "stacking" at the site of the immobilised IgG to assist in diagnosis or therapy of the patient by amplifying the concentration of the derivatised antibody that can be delivered to the site of the immobilised IgG. The present invention

further provides the above antibodies for use in a method of medical treatment.

The present invention is applicable to the diagnosis or treatment of conditions associated with immobilised IgG, and in particular agalactosyl IgG, such as autoimmune disorders including rheumatoid arthritis, juvenile arthritis, Crohn's disease, type I insulin dependent diabetes, type II diabetes, sarcoidosis, erythema nodosum leprosum and tuberculosis. In a preferred embodiment, the present invention can be employed in the diagnosis of erosive joint disease by detecting the presence of tissue immobilised anti-type II collagen antibodies produced in rheumatoid arthritis patients susceptible to erosive joint disease (Group 3).

In a further aspect, the present invention provides a method of producing a derivatised antibody, the method comprising treating a precursor antibody to expose carbohydrate chains so that the antibody is capable of binding to immobilised IgG. Preferably, the method involves thiolating the antibody in the presence of carbonate, such as ammonium carbonate or sodium carbonate. In some embodiments of the invention, only a fraction of the total derivatised antibodies can self associate or stack, referred to as the "imaging fraction" below. In this case, preferably the method additionally involves the step of separating this fraction of the derivatised antibodies, e.g. using Con A chromatography or by pH elution of the derivatised antibody from a Protein A affinity column. In a preferred embodiment,

the precursor antibody has Fc carbohydrate chains which terminate with N-acetylglucosamine (GlcNAc) residues. The proportion of such chains on the precursor antibody can be increased from naturally occurring levels by the use of β-galactosidase to remove terminal galactose residues. The imaging fraction of the derivatised antibodies should also contain Fab associated carbohydrate chains.

The method flips the internal carbohydrate chains, e.g. Fc carbohydrate chains, from their binding sites on the interstitial protein surface. The IgG is then chemically derivatised in a manner which prevents the carbohydrates from returning to their interstitial positions.

The binding between the immobilised IgG and the derivatised antibodies can be via the Fab carbohydrate chains of the derivatised antibodies binding to Fc binding sites or surfaces of the immobilised IgG and/or exposed Fab carbohydrate chains of the immobilised antibodies binding to Fc binding sites or surfaces exposed on the derivatised antibodies by the derivatisation reaction. Thus, in either case, the binding reaction is between a carbohydrate chain and a carbohydrate binding site on the antibody and is not linked to the immunological binding domain of the antibodies. In the case of agalactosyl IgG, as these antibodies lack galactosyl and sialic acid residues, this provides a binding site that can receive the available Fab carbohydrate chains of the derivatised antibodies. In preferred embodiment, the derivatised antibodies can

bind to themselves as well as binding with the immobilised IgG. This can happen if the flipping out of the carbohydrate chains opens up a binding site that is capable of binding carbohydrate chains on other antibodies (Fab, Fc etc). This stacking of the antibodies can provide a method of delivering the functional moiety to the locations in a patient where immobilised IgG concentrates, and also a method of concentrating the derivatised antibodies at these locations.

The precursor antibody can be polyclonal or monoclonal, and can be IgE, IgA, IgM (monomer), IgD or IgG. The production and purification of antibodies is described in detail below. In the examples below, the precursor antibody used is Sandoglobulin or CLB, polyclonal IgG mixtures.

In a further aspect, the present invention provides the use of a derivatised antibody for the preparation of a medicament for the treatment or diagnosis of a condition associated with immobilised IgG, the antibody being derivatised to expose carbohydrate chains so that the antibody is capable of binding to immobilised IgG.

In some embodiments, the derivatised antibody can be conjugated to a functional moiety, such as a label, toxin, drug, prodrug, effector or other moiety. Labels allow the antibody to be visualised *in vivo* or *in vitro*, indicating the presence and/or location of agalactosyl IgG. In a preferred embodiment, the label is a

radioactive label such as ^{99m}Tc. Where the functional moiety is a drug, prodrug or effector, the derivatised antibodies can deliver the functional moiety to locations in a patient's body where immobilised IgG is found and where the action of the drug, prodrug or effector is required.

Alternatively or additionally, the precursor antibody can itself have a therapeutic effect when delivered to locations in which immobilised IgG accumulates. By way of example, a derivatised antibody with specificity against an inflammatory mediator such as TNF-α could be used to ameliorate the effects of these substances at locations in a patient having elevated levels of immobilised IgG. In a further aspect, the present invention provides a method of diagnosing *in vivo* a condition associated with immobilised IgG, the method comprising (a) exposing a patient to derivatised antibodies, the antibodies being derivatised to expose carbohydrate chains so that the antibodies can bind to immobilised IgG and being labelled, and (b) using the label to detect the presence of the immobilised IgG. Conveniently, the derivatised antibodies can be administered to the patient by injection.

In a further aspect, the present invention provides a pharmaceutical composition comprising one or more of the above mentioned antibodies in combination with a pharmaceutically acceptable carrier.

The present invention will now be described by way of

example and not limitation with reference to the accompanying figures.

Brief Description of the Drawings

Figure 1 shows the anti-GlcNAc reactivity of immobilised Sandoglobulin or derivatised Sandoglobulin (HIG). Solutions containing various concentration of Sandoglobulin or HIG were added to microtitre wells coated with protein A. The top figure shows the anti-GlcNAc reactivity of the immobilised IgG or HIG after heating the plate 85°C for 10 minutes to mildly denature the protein and expose the carbohydrate residues. The bottom figure shows the anti-GlcNAc reactivity without the heating step.

Figure 2 shows the anti-GlcNAc reactivity of immobilised Sandoglobulin (top) or HIG (bottom). Solutions containing various concentrations of Sandoglobulin of HIG were added to micro titre plates directly/or to plates coated with protein A. Prior to the addition of the anti-GlcNAc monoclonal antibody the protein A coated plates were either heated to 85°C for 10 minutes or incubate at room temperature for a similar period.

Figure 3 shows the results of applying Sandoglobulin or HIG (4852) to an affinity column containing immobilised Concanavalin A. Three fractions were obtained: unbound, weakly bound - eluted with 100 MM α-methyl mannoside - and strongly bound - eluted with 100 mM HCl. Various concentrations of each of the three fractions were incubated in protein A coated microtitre wells. The

anti-GlcNAc reactivity of the immobilised Sandoglobulin of HIG fractions were measured. The bottom figure show the percentage of unbound and bound material for three different HIG preparations and underivatised Sandoglobulin.

Figure 4 shows anti-GlcNAc reactivity of immobilised CLB and derivatised CLB (HIG-62759) and Sandoglobulin and derivatised Sandoglobulin (HIG 4855). Solutions containing various concentrations of the different preparations were added to micro titre plates coated with protein A. The plates were then heated at 85°C for 10 minutes.

Figure 5 shows the anti-GlcNAc reactivity of immobilised Sandoglobulin derivatised with iminothiolane using different buffers. Solutions containing various concentrations of derivatised IgG were added to microtitre plates coated with protein A and then heated at 85°C for 10 minutes.

Figure 6(a) shows how the derivatised antibodies of the invention bind to anti-type II collagen IgG antibodies that are found immobilised in the joint of RA patients. Figure 6(b) shows the difference in the structure of carbohydrate chains in agalactosyl and normal antibodies

Figure 7 shows a schematic drawing of the species involved in the experiments shown in figure 3.

Figure 8 shows schematic drawing explaining how the

results from the stacking assays of figure 2 arise.

Figure 9 shows a graph of extent of denaturation plotted against temperature for HIG and Sandoglobulin, demonstrating how derivatisation leads to a greater degree of denaturation at a given temperature.

Detailed Description

Production of Antibodies

The precursor antibodies may be obtained commercially or prepared using techniques which are standard in the art. Human precursor antibodies can be obtained as commercially available polyclonal mixtures of antibodies such as Sandoglobulin or CLB, or obtained from large universal phage display libraries. Sandoglobulin is a polyclonal IgG/glucose preparation that is produced and commercially available from Sandoz. CLB is a polyclonal IgG preparation which can be obtained from the Central Laboratory of the Blood Transfusion Service of the Netherlands Red Cross, Amsterdam, Netherlands.

Derivatised antibodies made from human precursor antibodies have the advantage that they enable the use of repeat treatments due to the absence of the human anti-mouse antibody (HAMA) response (Schroff et al, Cancer Research, 45:879-885, 1985; DeJager et al, Proc. Am. Assoc. Cancer Res. 29:377, 1988). HAMA responses have a range of effects, from neutralisation of the administered antibody leading to a reduced therapeutic dose, through to allergic responses, serum sickness and renal impairment.

In some instances, antibodies of other species may be suitable precursor antibodies. Methods of producing such antibodies are very well known in the art and include immunising a mammal (e.g. mouse, rat, rabbit, horse, goat, sheep or monkey) with the protein or a fragment thereof. Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and screened, preferably using binding of antibody to antigen of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al, Nature, 357:80-82, 1992). Isolation of antibodies and/or antibody-producing cells from an animal may be accompanied by a step of sacrificing the animal.

In some embodiments, the precursor antibodies are monoclonal. Monoclonal antibodies can be made using the hybridoma method first described by Kohler et al, Nature 256:495, 1975, or made using recombinant means. Thus, an antibody specific for a protein may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, e.g. using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047. The library may be naive, that is constructed from sequences obtained from an organism which has not been immunised with any of the proteins (or fragments), or may be one constructed using sequences obtained from an organism which has been exposed to the antigen of interest.

Monoclonal antibodies can be subjected to the techniques of recombinant DNA technology to produce other antibodies

or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP 0184187 A, GB-A-2188638 or EP 0239400 A. A hybridoma producing a monoclonal antibody may be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced.

Pharmaceuticals

The antibodies of the invention can be formulated in compositions for therapeutic or diagnostic use. These compositions may comprise, in addition to one of the above substances, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes, and can be readily selected by those skilled in the art.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has

suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

In therapeutic embodiments, the derivatised antibodies are typically administered in an individual in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A. (ed), 1980.

Alternatively, targeting properties of the antibodies may be used to deliver the active agent more specifically to locations in a patient's body at which immobilised IgG, e.g. agalactosyl IgG, accumulates.

As mentioned above, the antibodies of the invention can be used to deliver one or more functional moieties to locations associated with immobilised IgG, the functional moiety being provided by the antibody itself or by the conjugation of the antibody to another molecule. An example of the former is the use of an antibody specific for an inflammatory mediator, such as tumour necrosis factor (TNFα). An example of the latter is conjugating the antibody to a precursor form of a drug, for conversion to the active form by an activating agent produced in, or targeted to, the cells to be treated. This type of approach is sometimes known as ADEPT which involves targeting the activating agent to the cells by conjugation to a cell-specific antibody, see for example EP 0415731 A.

Materials and Methods

1. Production of Monoclonal Antibody 3C4

An IgM monoclonal (3C4) with specificity towards terminal N-acetylglucosamine (GlcNAc) was produced by the UCL Monoclonal Antibody Unit by *in vitro* culture. Initial screening of clones used terminal N-acetylglucosamine rich ovalbumin and then agalactosyl IgG. The 3C4 monoclonal was purified from culture supernatant using GlcNAc-sepharose® and eluted with 0.5M GlcNAc. It was then dialysed and biotinylated. This antibody was used as a probe for GlcNAc residues in the experiments described below.

2. 3C4 Stacking Assays

(*i*) Nunc® maxi-sorp plates were coated with 50µl of

Protein A at 2.5mg/ml in PBS overnight at 4°C. Plates were washed with PBS/0.05%Tween® 20 (PT) and blocked with 100µl of 1% BSA in PT (PBT) for 1h at 37°C. The plates were then washed with PT, and the human IgG to be tested was added in 100mM glycine/150mM NaCl pH 8.2 buffer as serial five fold dilutions in triplicate starting at 20µg/well in 50µl (400µg/ml) for six dilutions such that the lowest amount was around 6ng/well1. The plates were then incubated for 2h at 37°C, and then washed five times with PT. 100µl of PBS was added to each well and the plates were then floated on a water bath at 85°C for 15 minutes to denature the IgG and expose the Fc N-linked oligosaccharides. The PBS was shaken out and 125ng of biotinylated 3C4 monoclonal was then added per well in PBT and incubated overnight at 4°C. Following five further washes visualisation of the bound 3C4 was achieved by addition of 0.1mg 2,2'-azino-bis[3-ethylbenz-thiazoline-6-sulphonic acid] in 50µl 0.1M citrate phosphate, pH 4.1 containing 0.005% hydrogen peroxide, and incubation in the dark for 10 minutes at room temperature. The reaction was stopped by the addition of 50µl of 0.2% sodium fluoride and absorbance measured at 650nm-490nm.

(ii) As a variation on the above assay, human IgG was also coated directly to Nunc® Maxi-sorp plates in 50µl PBS at the same serial dilutions 20µg/well to 6ng/well as stated above. Efficient coating was achieved after 2h at 37°C. From this point the assay was identical to the one above from the heating step, i.e. 100µl of PBS was added to each well and the plates were then floated on a water

bath at 85°C for 15 minutes. This assay gave higher levels of bound human IgG and differs from the previous assay with respect to the orientation of the initial IgG monolayer.

3. Thiolation of Human IgG

Human IgG (30mg) was dissolved in 0.5ml of 15mM ammonium bicarbonate, with all solutions being prepared and kept oxygen free by using degassed solvent and being maintained under nitrogen. 0.63mg of 2-iminothiolane-HCl in 1 ml of 15mM ammonium bicarbonate was added and mixed gently on a roller device for 20 minutes. The derivatised IgG was then separated from the excess 2-iminothiolane using a disposable Sephadex® G-25 (PD10) column that had been blocked with 1% human serum albumin in 0.9% NaCl, followed by equilibration in 15mM ammonium bicarbonate. The elution of the derivatised human IgG was achieved with 15mM ammonium bicarbonate and monitored by 280nm absorbance. The tin/tartrate solution was made as follows. Di-sodium tartrate (4mg/mg IgG) was dissolved in 10ml of sterile oxygen free water. 4µg of tin (II) chloride per mg IgG was added to the solution (stock solution 750µg Sn^{2+}/mol in 1% HCl).

The IgG column eluate was added to the 10 ml solution of tartrate and tin and brought to a final concentration of 1mg IgG/ml using the derivatisation buffer.

4. Preparation of Concanavalin A Positive Human IgG

A 2ml bed volume column of Con A Sepharose® (Sigma) was equilibrated with 50mM Tris pH 7.2 with 200mM sodium

chloride and 2mM $MnCl_2$, $CaCl_2$. 10mg of human immunoglobulin that had previously been dialysed and freeze dried was dissolved in 1ml of the above buffer and the applied to and then allowed to sit on the column for 10 minutes at room temperature. The column was then washed with several column volumes of the above buffer and the Con A unbound IgG fraction eluted and monitored by 280nm absorbance. The Con A positive fraction was then recovered by elution with 5 column volumes of 100 mM HCl and then the pH was returned back to neutrality as soon as possible with a minimal volume of a 1M Tris solution. The absorbance of the bound fraction was measured at 280nm, it was usually found that approximately 25% of the IgG was Con A positive. Both fractions were dialysed against water and freeze dried prior to further analysis.

Results and Discussion

Figure 1, top, shows that the anti-GlcNAc reactivity of derivatised IgG (HIG) as described in method (3) is greater than the starting material (Sandoglobulin) at any fixed concentration. Since both the Sandoglobulin and the HIG have the same chemical content of terminal N-acetyglucosamine residues on the carbohydrate chains attached to the IgG molecules, respectively, the increase in the reactivity must result from an increase in accessibility of the carbohydrate residues to the 3C4 anti-GlcNAc monoclonal probe.

The carbohydrate residues on the Fc fragment IgG are normally inaccessible since they are buried inside the

protein (see Figure 7). By heating to 85°C, some of the carbohydrates chains become accessible and can then be detected by 3C4. The increase in accessible carbohydrate chains for HIG, when compared to Sandoglobulin, suggests that at 85°C the HIG is more easily thermally denatured, exposing a greater number of chains. This is shown in Figure 9, which shows a graph showing how the accessibility of GlcNAc residues of HIG and the Sandoglobulin starting material varies with temperature. This demonstrates that the derivatisation reaction reduces the temperature at which the carbohydrate chains become available towards physiological temperature (see Figure 1, bottom). This shift is particularly advantageous as it provides a method of derivatising the antibodies while avoiding loss of the antibody binding activity caused by thermal denaturation, e.g. making it possible to make derivatised antibodies that retain a useful activity associated with the antibody binding reaction, such as binding specificity for an inflammatory mediator. The use of thermal denaturation to "flip out" the sugars is not a useful method since (1) on returning to physiological temperatures the sugars will flip back in and (2) the thermal denaturation may disrupt the antigen binding site on the antibody. Accordingly, the two step method for producing derivatised antibodies described in this embodiment provides (1) a chemical denaturant to "flip out" the sugar chains at physiological temperatures followed by (2) a chemical derivatisation to prevent them from flipping back.

When adding increasing amounts of IgG to a microtitre

well saturation would be expected. Figure 2 shows that whereas Sandoglobulin saturates, there is a continual increase in anti-GlcNAc reactivity of the HIG as the concentration of HIG applied to the microtitre plates is increased. This indicates that a new top surface monolayer is being formed for the case of HIG which contains increasing proportions of anti-GlcNAc reactive IgG. This "stacking" of anti-GlcNAc reactive IgG onto the initial Protein A immobilised monolayer and subsequent monolayers is a property unique to the HIG and not found for Sandoglobulin. This phenomenon is described in Figure 8, which show how the proportion of derivatised antibody (shown by the filled circles), as compared to the immobilised IgG (shown by the open circles), increases in successive layers of the stack.

Figure 2 demonstrates that the stacking of anti-GlcNAc reactive IgG also occurs when the initial monolayer is not oriented by the coating of the plate (i.e. Protein A). Figure 2 also demonstrates that even in the absence of heating there is a significant amount of anti-GlcNAc reactive IgG in the HIG preparation and that essentially none exists in the Sandoglobulin.

IgG is also known to contain carbohydrate residues in the Fab region of the molecule. The Fab carbohydrates are accessible to lectin binding and therefore IgG molecules containing Fab associated carbohydrates can be separated by affinity chromatography. Figure 3 (bottom) shows that the percentage of Con A bound (IgG containing Fab carbohydrates) and Con A unbound (IgG with no Fab

carbohydrates) is the same for Sandoglobulin and HIG. Figure 3, top, however shows that all of the "stacking" IgG is found in the Con A bound fraction of HIG. These species are shown schematically in Figure 7. Figure 3 also suggests that in cases in which not all of the derivatised antibodies are capable of stacking, a separation step should be employed to separate the stacking antibodies.

These results suggest that the "stacking" IgG has three specific characteristics:

(a) Firstly, "stacking" HIG is reactive with anti-GlcNAc monoclonal antibodies which are probing the carbohydrates on the Fc fragment.

(b) Secondly, it is only those HIG molecules which also have a Fab associated carbohydrate which stack.

(c) Thirdly, the increased proportion of anti-GlcNAc reactive material which is found when the HIG concentration is increased indicates that the "stacking" IgG prefers to stack itself (i.e. self-association).

In patients with rheumatoid arthritis, there is an increase in the serum content of agalactosyl IgG glycoform. This glycoform is disease associated and is characterised by having an increased proportion of its Fc-associated carbohydrate chains terminating with N-acetylglucosamine rather than galactose or sialic acid, see Figure 6(b). This form of IgG therefore has an

increased anti-GlcNAc reactivity with the 3C4 monoclonal similar to the *in vitro* produced HIG. Immobilised agalactosyl IgG will therefore act as a preferential surface for the "stacking" of HIG *in vivo*. In this way, HIG is able to selectively bind to tissue surfaces (e.g. synovial joints) of patients with autoimmune disorders in the presence of large concentrations of endogenous IgG.

Figures 4 and 5 demonstrate that the iminothiolane derivatisation reaction itself is not sufficient to produce HIG which is able to stack. This result is unexpected and novel. The most probable mechanism is that certain buffers (e.g. ammonium bicarbonate) are able to briefly cause the carbohydrate residues to "flip out", exposing cryptic amino acid residues which can then be derivatised with the iminothiolane. Once derivatised, the presence of the iminothiolane groups sterically prevent the carbohydrate chains from flipping back inside the protein, see Figure 7(b). An alternative mechanism is that when the carbohydrate chains are "flipped out", the iminothiolane derivatisation of amino acid residues remote from the carbohydrate binding site results in stabilisation of a protein conformation in which the carbohydrate chains spend more time "flipped out" at 37°C (see Figure 1(b)). That is, the dynamic equilibrium is shifted such that the sugar chains become more mobile at body temperature and consequently the protein surface becomes exposed for a greater time for interaction with carbohydrate chains from adjacent molecules.

Figure 6(a) shows how the derivatised antibodies of the

invention bind to anti-type II collagen IgG antibodies that are found immobilised in the joint of RA patients. The derivatised antibodies have flipped out carbohydrate residues that are available to bind to the anti-type II collagen IgG antibodies, and have binding sites opened up for binding by the agalactosyl carbohydrate chains of the immobilised IgG. This means that the derivatised antibodies can bind to sites where the immobilised antibodies are located and stack to provide amplification of a label for diagnosis or another kind of effector moiety for a therapeutic purpose.

Figure 6(b) shows the difference in the structure of carbohydrate chains in agalactosyl and normal antibodies, the agalactosyl IgG missing galactosyl and sialic acid residues from the terminus of the carbohydrate chain.

Figure 7 shows a schematic drawing of the species involved in the experiments investigating the stacking properties of the HIG derivatised antibodies described above. Thus, the derivatisation reaction flips out the Fc carbohydrates (compare Figure 7 Con A bound and Figure 7 (HIG)) that were bound to the internal protein surface. This surface (binding site) is now vacant and can interact with another carbohydrate chain on an adjacent IgG molecule. The data in Figure 3 indicates that this carbohydrate chain is the one attached to the Fab (see Figure 7 Con A bound) of an adjacent IgG molecule. Serum agalactosyl IgG (Figure 6(b)) behaves like the derivatised antibodies since it is missing carbohydrate residues exposing the same protein surface which occurs

in derivatised IgG when the carbohydrate chains flip out.

The references mentioned herein are all expressly incorporated by reference.

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CLAIMS:

1. A method of producing a derivatised antibody so that the antibody is capable of binding to immobilised IgG, the method comprising:

treating a precursor antibody to expose a carbohydrate chain of the precursor antibody from an interstitial site on the surface of the antibody; and,

chemically derivatising the antibody to prevent the carbohydrate chain returning to the interstitial site so that the antibody is capable of binding to the immobilised IgG.

2. The method of claim 1, wherein the precursor antibody has Fc associated carbohydrate chains which terminate with an N-acetylglucosamine residue.

3. The method of claim 1 or claim 2, wherein the method includes the step of using ß-galactosidase to remove terminal galactose residues from the precursor antibody.

4. The method of any one of claims 1 to 3, wherein the chemical derivatisating step comprises thiolating the antibody in the presence of carbonate.

5. The method of any one of the preceding claims, wherein the method includes the step of separating derivatised antibodies which can associate with one another at a site of immobilised IgG, from those derivatised antibodies which cannot so associate.

6. The method of claim 5, wherein said step of separating the derivatised antibodies is by the use of Con A chromatography or by pH elution from a Protein A affinity column.

7. An antibody as obtainable by the method of any one of the preceding claims, wherein the antibody has been derivatised to expose a carbohydrate chain thereof so that the antibody is capable of binding to immobilised IgG.

8. The antibody of claim 7, wherein the carbohydrate chain is a Fc carbohydrate chain.

9. The antibody of claim 7 or claim 8, wherein the carbohydrate chain terminates with an N-acetylglucosamine residue.

10. The antibody of any one of claims 7 to 9, wherein the carbohydrate chains of the derivatised antibody are capable of specifically binding a binding site on the immobilised antibody.

11. The antibody of any one of claims 7 to 10, wherein the immobilised IgG is agalactosyl IgG.

12. The antibody of any one of claims 7 to 11, wherein the antibody is conjugated to a label, toxin, drug, prodrug or effector.

13. The antibody of any one of claims 7 to 12, wherein the antibody is specific for an inflammatory mediator.

14. The antibody of any one of claims 7 to 13 for use in a method of medical treatment or diagnosis of a condition associated with immobilised IgG.

15. The antibody of claim 14 for use in the diagnosis or treatment of an autoimmune disorder.

16. The antibody of claim 15, wherein the autoimmune disorder is selected from rheumatoid arthritis, juvenile arthritis, Crohn's disease, type I insulin dependent diabetes, type II diabetes, sarcoidosis, erythema nodosum leprosum and tuberculosis.

17. The antibody of claim 16, wherein the antibody is for use in the diagnosis or treatment of erosive joint disease and the immobilised IgG is anti-type II collagen IgG.

18. Use of a derivatised antibody of any one of claims 7 to 14 for the preparation of a medicament for the treatment or diagnosis of a condition associated with immobilised IgG.

19. The use of claim 18, wherein the condition associated with immobilised IgG is an autoimmune disorder.

20. The use of claim 16, wherein the autoimmune disorder is selected from rheumatoid arthritis, juvenile arthritis, Crohn's disease, type I insulin dependent diabetes, type II diabetes, sarcoidosis, erythema nodosum leprosum and tuberculosis.

21. The use of claim 20, wherein the antibody is for use for the diagnosis or treatment of erosive joint disease and the immobilised IgG is anti-type II collagen IgG.

22. Use of a derivatised antibody of any one of claims 7 to 14 for the *in vivo* diagnosis of a condition associated with immobilised IgG, wherein the use comprises:

(a) exposing a patient to said antibody which has been labelled; and,

(b) using the label to detect the presence of the immobilised IgG.

23. A pharmaceutical composition comprising a derivatised antibody of any one of claims 7 to 14, in combination with a carrier.

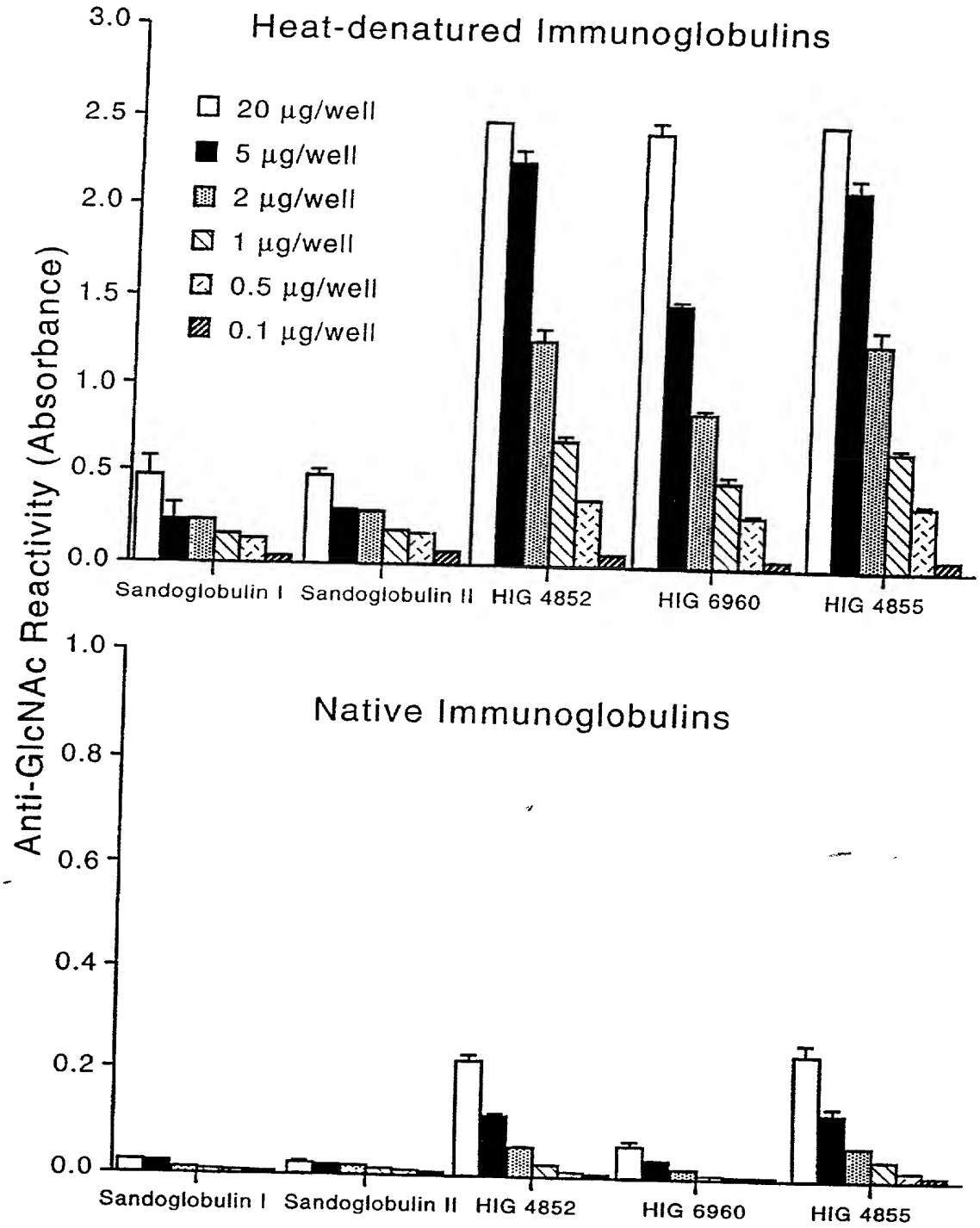

Heat-denatured Immunoglobulins
20 µg/well
5 µg/well
2 µg/well
1 µg/well
0.5 µg/well
0.1 µg/well
Anti-GlcNAc Reactivity (Absorbance)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Sandoglobulin I
Sandoglobulin II
HIG 4852
HIG 6960
HIG 4855
Native Immunoglobulins
1.0
0.8
0.6
0.4
0.2
0.0
Sandoglobulin I
Sandoglobulin II
HIG 4852
HIG 6960
HIG 4855

Figure 1

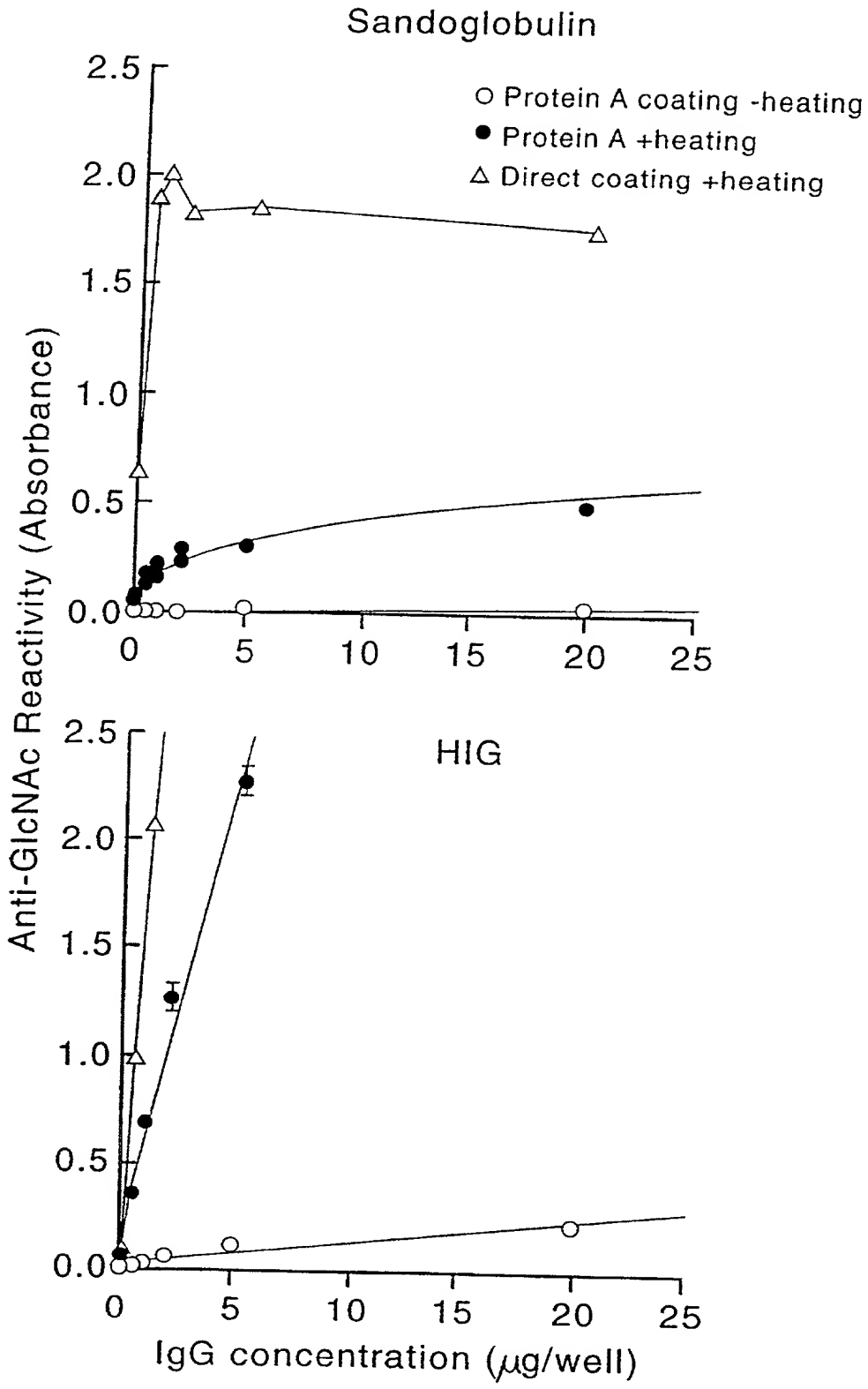

Sandoglobulin
Protein A coating -heating
Protein A +heating
Direct coating +heating
Anti-GlcNAc Reactivity (Absorbance)
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
HIG
IgG concentration (μg/well)

Figure 2

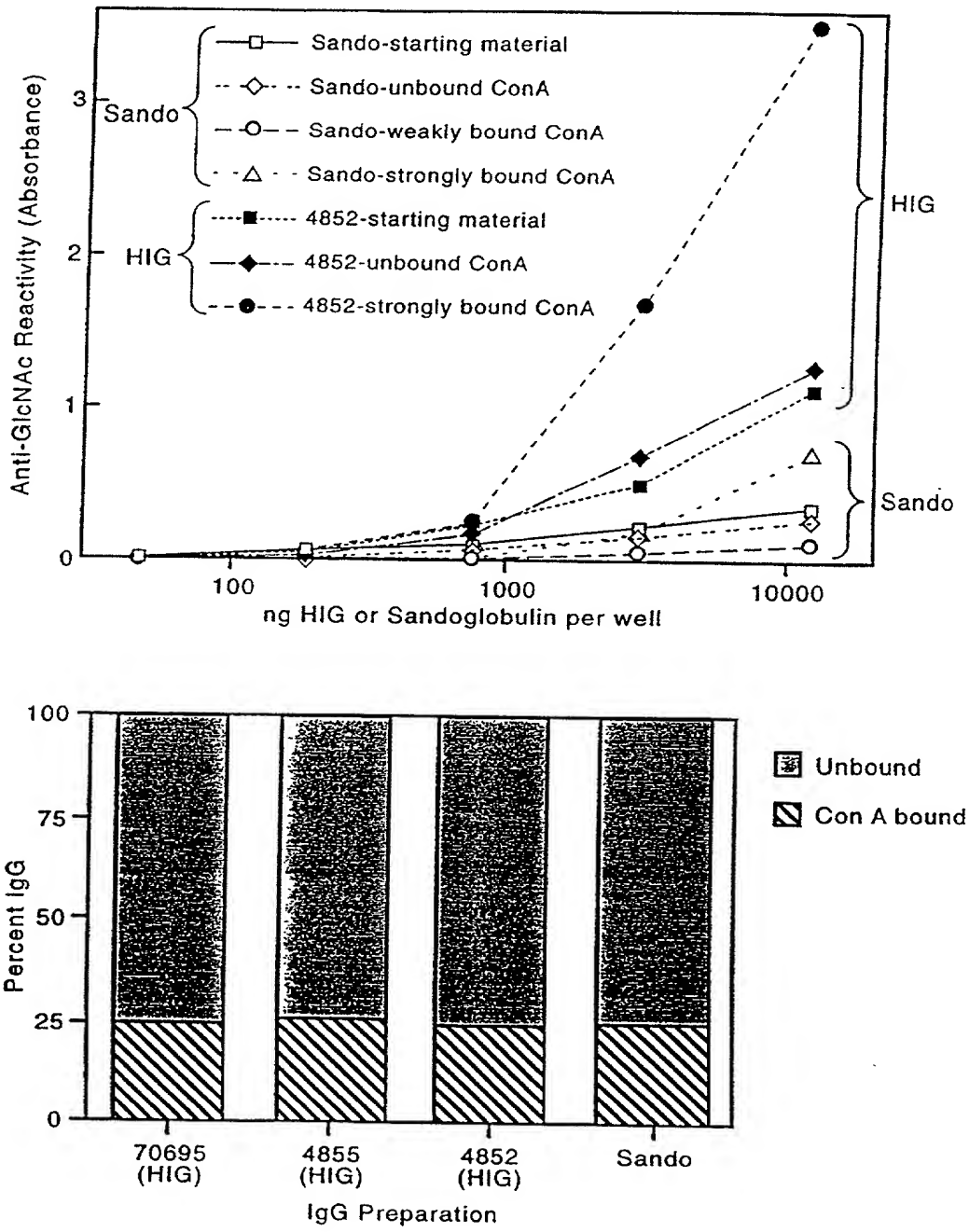

Sando-starting material
Sando-unbound ConA
Sando-weakly bound ConA
Sando-strongly bound ConA
4852-starting material
4852-unbound ConA
4852-strongly bound ConA
Sando
HIG
Anti-GlcNAc Reactivity (Absorbance)
0
1
2
3
100
1000
10000
ng HIG or Sandoglobulin per well
HIG
Sando
Unbound
Con A bound
Percent IgG
0
25
50
75
100
70695 (HIG)
4855 (HIG)
4852 (HIG)
Sando
IgG Preparation

Figure 3

Anti-GlcNAc Reactivity (Absorbance)
2.0
1.5
1.0
0.5
0.1
CLB-IgG
HIG-62759
Sando-IgG
HIG-4855
1
10
µg of IgG/well
1.00
0.75
0.50
0.25
1µg/well
A
B
C
D
E
F
G
H
I
J
A=HIG 4852 control
B=Ammonium bicarbonate
C=Sodium bicarbonate
D=Neostigmine
E=Urea
F=DTPA
G=Guanidine
H=Sandoglobulin control
I=SDS
J=HSA control

Figure 4

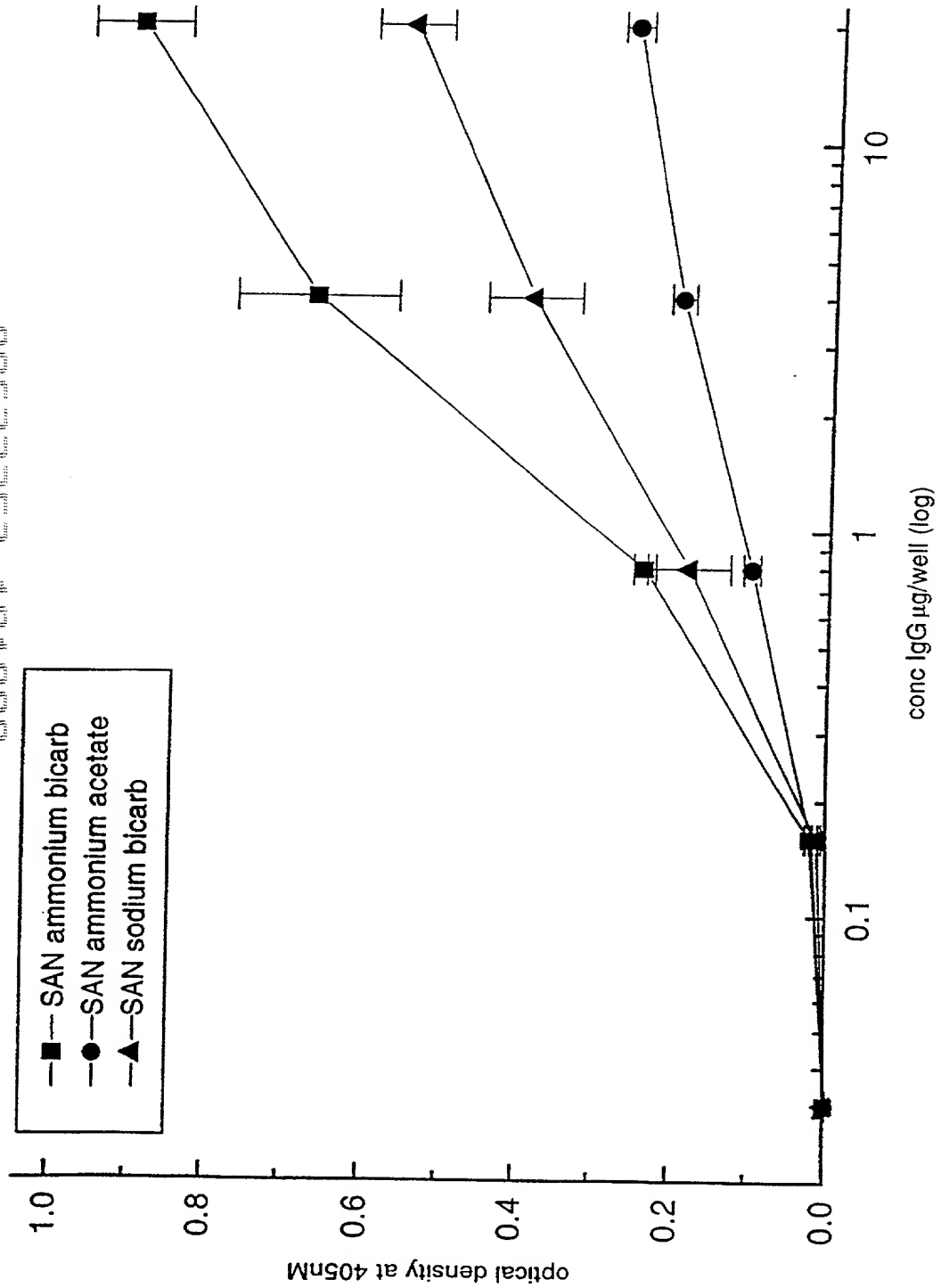

optical density at 405nM
1.0
0.8
0.6
0.4
0.2
0.0
0.1
1
10
conc IgG µg/well (log)
SAN ammonium bicarb
SAN ammonium acetate
SAN sodium bicarb

Figure 5

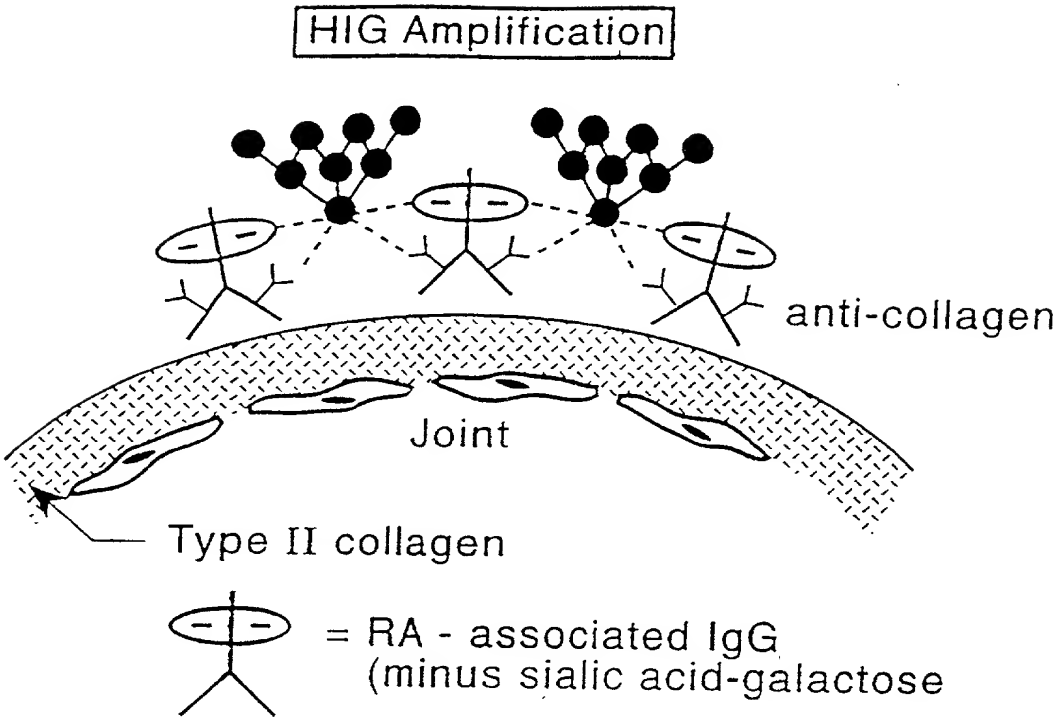


Figure 6(a)

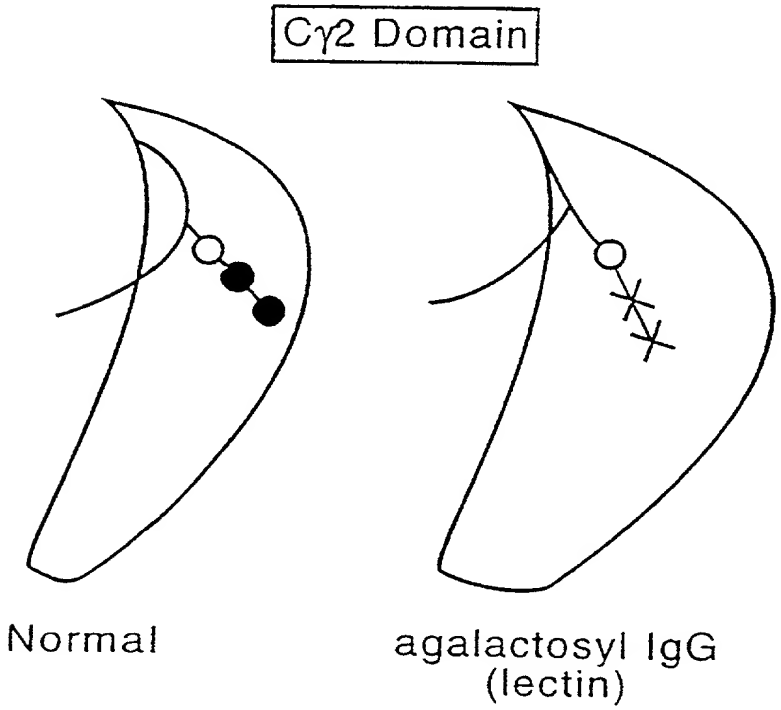


Figure 6(b)

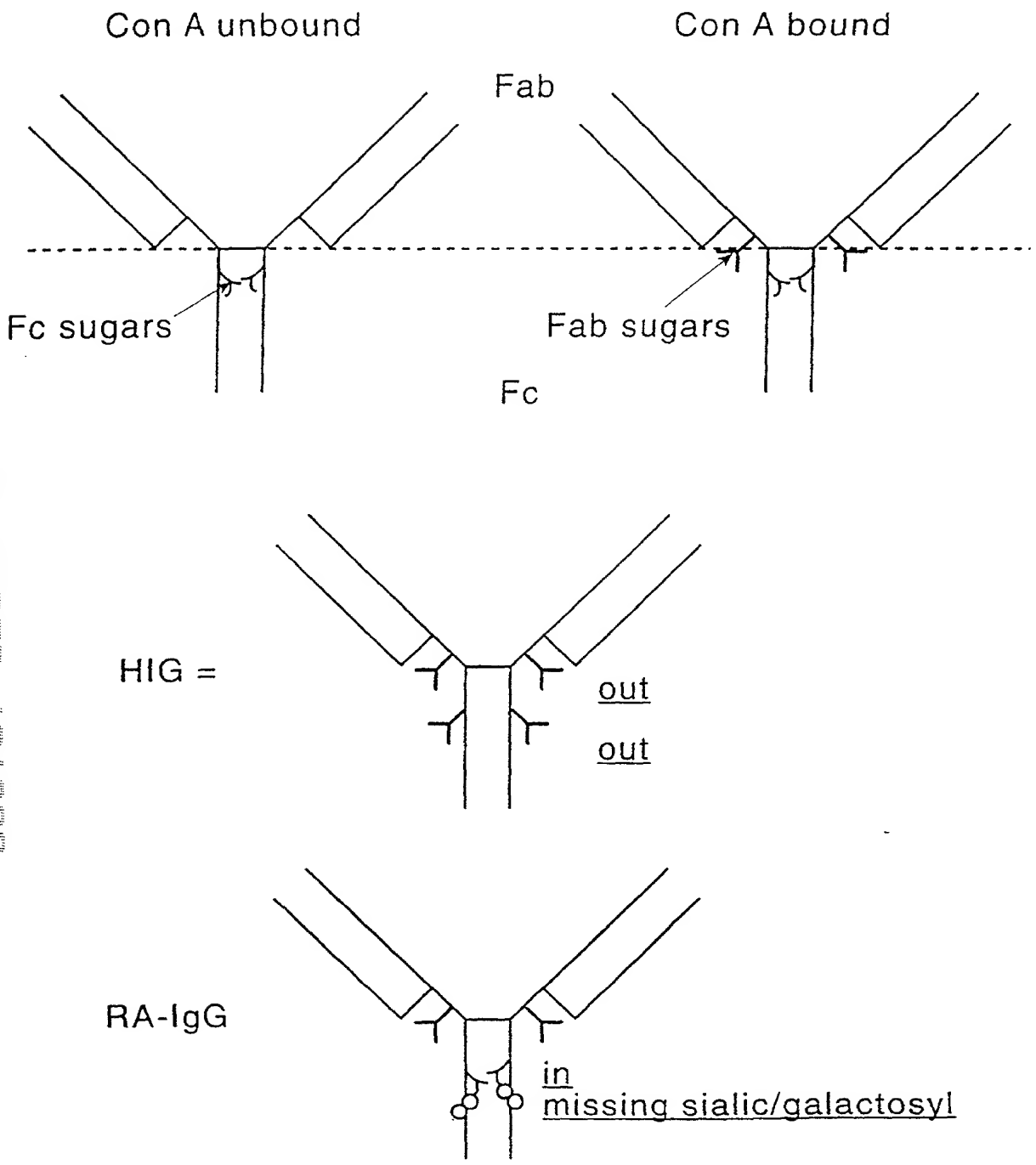

Con A unbound
Con A bound
Fab
Fc sugars
Fab sugars
Fc
HIG =
out
out
RA-IgG
in
missing sialic/galactosyl

Figure 7

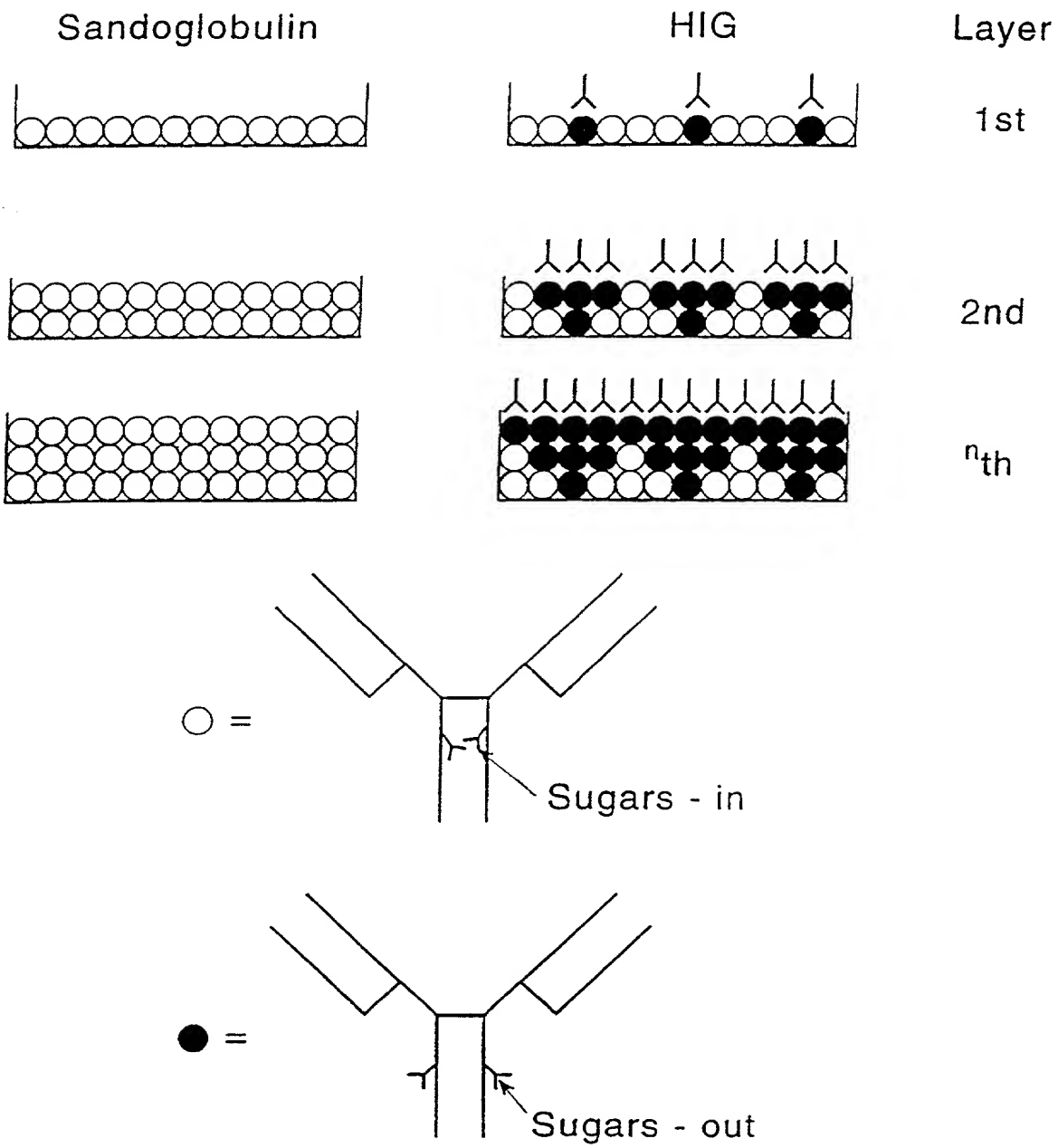
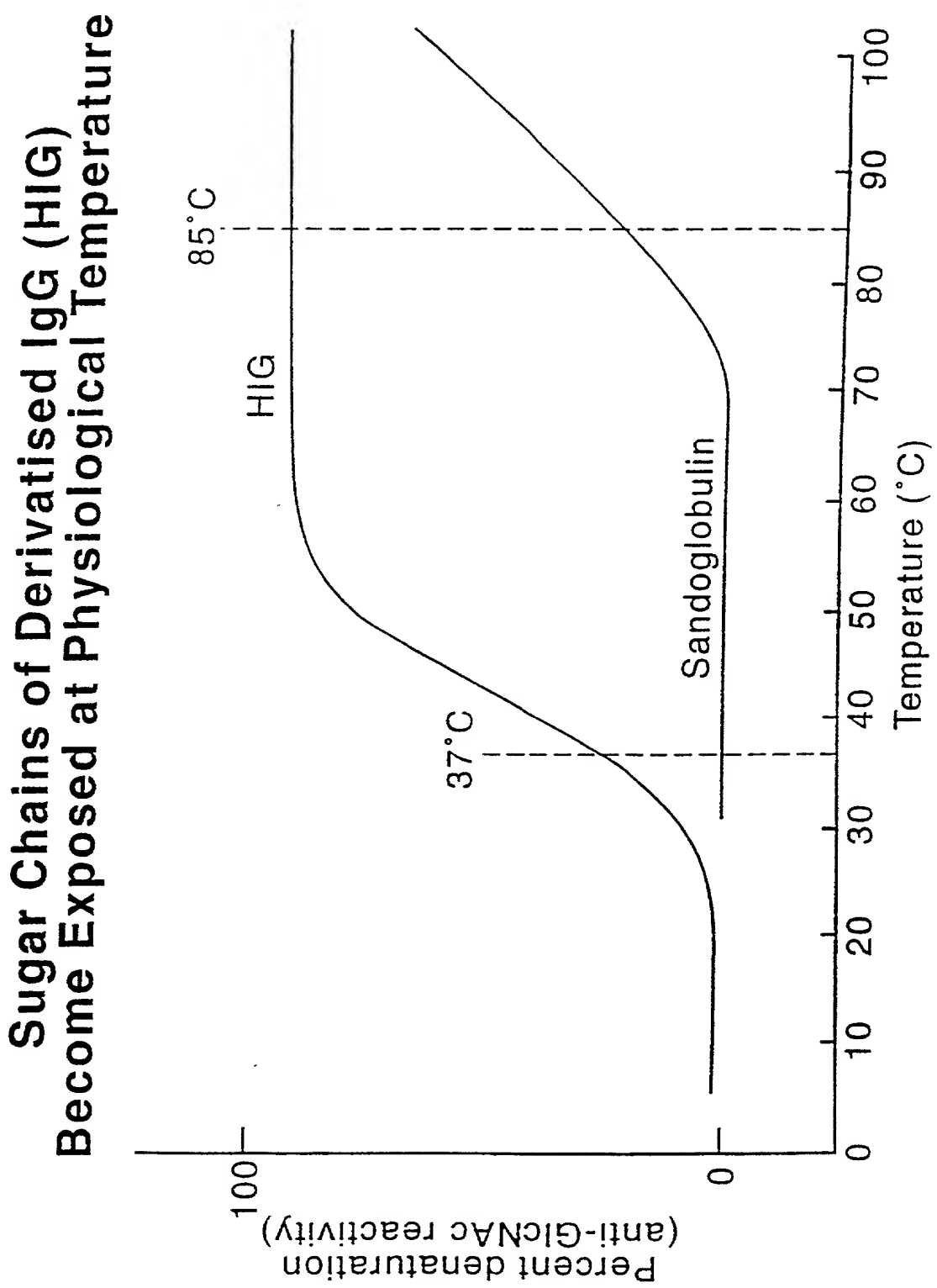

Sandoglobulin
HIG
Layer
1st
2nd
nth
○ =
Sugars - in
● =
Sugars - out
人 = anti-GlcNAc antibody

Figure 8

Figure 9


Sugar Chains of Derivatised IgG (HIG)
Become Exposed at Physiological Temperature
85°C
HIG
37°C
Sandoglobulin
100
0
Percent denaturation
(anti-GlcNAc reactivity)
0
10
20
30
40
50
60
70
80
90
100
Temperature (°C)

DECLARATION

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **DERIVATISED ANTIBODIES WITH EXPOSED CARBOHYDRATE CHAINS CAPABLE OF BINDING TO IMMOBILISED IGG** the specification of which __ is attached hereto or ____ was filed on August 15, 2000, as Application No. 09/622,253 , and was amended on _____ (if applicable).

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56. I claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Country	Application No.	Date of Filing	Priority Claimed Under 35 USC 119
GB	9803257.6	16 February 1998	Yes
PCT	PCT/GB99/00385	05 February 1999	Yes

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date

I claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Date of Filing	Status

1-00

Full Name of Inventor 1:	Full Name: **RADEMACHER, Thomas William**		
Residence & Citizenship:	City: **Boars Hill**	State/Foreign Country: **United Kingdom** GBX	Country of Citizenship: **GB**
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Full Name of Inventor 2:	**WILLIAMS, Phillip**		
Residence & Citizenship:	City: **Botley**	State/Foreign Country: **United Kingdom** GBX	Country of Citizenship: **GB**
Post Office Address:	Post Office Address: **29 Eynsham Road, Botley, Oxford OX2 9B5 United Kingdom**		

2-00

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 1	Signature of Inventor 2	
RADEMACHER, Thomas William	**WILLIAMS, Phillip**	
Date 15/09/00	Date 11/9/00	